Identification of seed-borne *Pea seed-borne mosaic virus* in lentil (*Lens culinaris*) germplasm and strategies to avoid its introduction in commercial Australian lentil fields

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Abstract *Pea seed-borne mosaic virus* (PSbMV) is not considered a problem in Australian lentils as no seed transmission in commercial lentil seed lots has so far been reported. PSbMV lentil-seed transmissible strains were found in accessions from the Australian germplasm collection that were imported from the USDA germplasm collection and were previously reported to contain PSbMV. Strategies to clean up infected entries and to avoid the spread of these strains to commercial fields are discussed.

Keywords Potyvirus · Biosecurity · Germplasm conservation · PSbMV

Pea seed-borne mosaic virus (PSbMV, genus *Potyvirus*, family *Potyviridae*) is a non-persistently transmitted virus that has the potential to spread rapidly and cause serious losses to grain yield and quality in field pea (*Pisum sativum*, Coutts et al. 2009) as well as lentils (*Lens culinaris*, Kumari and Makkouk 1995). PSbMV has a narrow host range, restricted to a limited number of *Fabaceae* species (Aapola et al. 1974). In the absence of alternative hosts, epidemic development of PSbMV in commercial crops has to rely on seed transmission to survive crop free periods. In Australia, very high levels of PSbMV seed transmission have been found in several field pea cultivars (Coutts et al. 2009; van Leur et al. 2013) and incidences in pea crops were found to

be related to infection rates in the seed used, both in experiments (Coutts et al. 2009) and in commercial fields (Freeman et al. 2013). Low levels of transmission through lentil seed has been found under experimental conditions (Coutts et al. 2008), but not in commercial seed lots and PSbMV plant infection in lentil fields is invariable related to nearby infection in pea fields (Aftab and Freeman, unpublished results). A similar situation—high levels of PSbMV in susceptible pea cultivars, but its absence in commercial lentil fields—is as well present in the USA (Goodell and Hampton 1984) and Canada (Howard et al. 1981), where lentil is a relatively young crop like Australia. This is in stark contrast with countries in the old world that have a long tradition of lentil cultivation: In Ethiopia, Abraham and Makkouk (2002) examined 270 lentil seed lots and found PSbMV seed transmission in 31 % of the lots, with up to 17 % transmission levels. Also in Ethiopia, Tadesse et al. (1999) found an average 10 % plant infection in 32 lentil fields (range 0–58 %) and Bekele et al. (2005) recorded an average of 52 % plant infection in 8 lentil fields. High levels of plant infection in lentil fields were as well reported in Syria (Makkouk et al. 1992), Pakistan (Makkouk et al. 2001) and Azerbaijan (Mustafayev et al. 2011).

Lentils have been grown on small scale in Australia since the 1980s, but large scale cultivation only started after a breeding program was initiated in 1994 (Brouwer 2002). To enable selection of locally adapted cultivars, a large number of germplasm accessions was imported from the USDA collection and other sources and deposited with the Australian Temperate Field Crop Collection (ATFCC) at Horsham. Part of this collection was imported into Australia without checking for seed-borne viruses, as lentils were only formally subjected to quarantine inspection since 2004 and is therefore a potential source of exotic PSbMV strains. PSbMV infection in the USDA lentil germplasm collection has been reported previously: Hampton (1982) evaluated 570 accessions by assaying seedlings on *Chenopodium*

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amaranticolor and found 38 to transmit PSbMV. More PSbMV contaminated lines were identified in this collection using Enzyme-linked immunosorbent assay (ELISA) by Goodell and Hampton (1984), who also flagged the danger of spread of lentil seed-transmissible virus strains from the germplasm collection to the commercial lentil industry in the USA.

We identified 24 accessions in the ATFCC lentil germplasm collection that were imported from the USDA collection and had been identified as PSbMV contaminated by Hampton (1982) and/or Goodell and Hampton (1984). Our aim was to check these accessions for PSbMV and to minimise the chance of lentil-specific strains spreading to commercial crops by purifying infected accessions. Depending on seed availability, up to 100 seed/accession were tested for PSbMV seed transmission. Small (5×5×12 cm) pots were sown with up to 4 seed in an aphid proof, temperature controlled (18–24°) greenhouse at the Tamworth Agricultural Institute during 2012. Four weeks after sowing all

emerged seedlings were tested for the presence of PSbMV, individually by Tissue blot immunoassay (TBIA) and in groups of 5–20 plants (depending on the number seed sown/pot) by Reverse-transcriptase polymerase chain reaction (RT-PCR) using procedures described in detail by Freeman et al. (2013). Out of the 24 tested accessions, 5 were found to contain PSbMV transmitting seed (Table 1). TBIA and RT-PCR yielded the same results, but TBIA had the advantage of being able to identify individual PSbMV-infected seedlings and thereby allowing a more precise estimation of infection incidence. However, RT-PCR will be more suitable to test bulk samples of large numbers of plants. PSbMV-free seed of these accessions was produced by selecting pots of which none of the seedlings were found to be PSbMV infected and transplanting the plants in larger pots to grow to maturity.

PSbMV isolations were made from TBIA identified positive seedlings by homogenising the seedlings in a cold pH neutral phosphate buffer and rubbing the suspension into

Table 1 PSbMV infection incidence in lentil germplasm accessions imported from the USDA collection and reported (Hampton (1982), Goodell and Hampton (1984)) to be infected with PSbMV. Number of isolates made and pathotyped

ATC ^a	PI ^b	Origin	Number of tested seedlings	PSbMV positive seedlings ^c	% infection	Number of isolates made	Pathotyped isolates
71095	212609	Afghanistan	76		0		
70951	320945	Bulgaria	78	1	1		
71307	299222	Chile	77	5	6	4	4
71315	299233	Chile	97	8	8	5	4
71342	299265	Chile	75		0		
71389	299318	Chile	76		0		
71080	185602	Egypt	57		0		
71146	289077	Egypt	96		0		
71163	297746	Greece	61		0		
71169	297752	Greece	76		0		
71189	297772	Greece	98	10	10	7	4
71743	431655	Iran	25		0		
72165	432077	Iran	54		0		
72200	432112	Iran	22		0		
72209	432121	Iran	76		0		
72214	432126	Iran	55		0		
72306	432218	Iran	93	4	4	4	4
71244	299127	Mexico	78		0		
70278	374120	Morocco	76		0		
71116	251784	Russian Federation	74		0		
71015	176604	Turkey	53		0		
71021	176988	Turkey	52		0		
71058	179319	Turkey	75		0		
71104	244046	Yemen	70		0		

^a Accession code Australian Temperate Field Crop Collection

^b Accession code USA Department of Agriculture Germplasm Collection

^c PSbMV infection determined by Tissue blot immunoassay

carborundum dusted first and second leaves of three 2-wk old faba bean (*Vicia faba*, cv. 'Fiesta') plants. PSbMV infection of the inoculated faba beans was checked by TBIA 2–3 weeks after inoculation. Young leaves harvested from virus positive faba bean plants were used for pathogenicity tests and to preserve isolates for further studies. Out of a total of 20 isolates from 4 accessions (Table 1), 16 were tested for pathogenicity using the PSbMV strain differentiating pea lines PI 269774 and Dark Skin Perfection (Johansen et al. 2001), as well as two PSbMV susceptible check lines; the pea variety 'Kaspa' and the lentil variety 'Nipper'. First and second leaves of a minimum of 5 plants per entry were inoculated 10–14 days after sowing with homogenised fresh leaves of the faba bean increase hosts. The inoculation was repeated on the third leaf after 1 week. Two weeks after the second inoculation the youngest emerged leaves of the inoculated plants were TBIA tested for virus presence. None of the 16 tested isolates infected the two PSbMV differentials, while all showed a near 100 % infection on the two susceptible check lines. The isolates can therefore be classified as the P2 pathotype, similarly to the L-1 lentil-derived strain pathotyped by Alconero et al. (1986). Only limited surveys for PSbMV pathotypes isolated from field pea have been carried out in Australia, but this pathotype has so far not been identified (Torok and Randles 2007).

The presence of PSbMV strains that can be transmitted through lentil seed is of great danger to the emerging Australian lentil industry. PSbMV infected accessions, imported into Australia before proper quarantine procedures were in place, have been increased in the field, thereby potentially spreading the virus to other accessions or commercial lentil fields. To check whether infection had occurred during these increases, we tested 5 accessions that were grown in the field at Horsham in 2000 next to accessions we found to be PSbMV infected: ATC71313 (PI 299230, originating from Chile) and ATC71316 (PI 299234, Chile) bordered ATC71315 (PI 299233); ATC71186 (PI 297769, Greece) bordered ATC71189 (PI 297772); ATC72305 (PI 432217, Iran) and ATC72307 (PI 432219, Iran) bordered ATC72306 (PI 432218). From each of the 5 accessions 90–100 seedlings were tested by both TBIA and RT-PCR, but no PSbMV infection was found. Nevertheless caution has to be exercised with increasing lentil accessions in the future and a routine testing program to test bulk samples by RT-PCR for PSbMV presence will be put in place. Accessions found to be PSbMV contaminated will be cleaned using the methods outlined above.

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